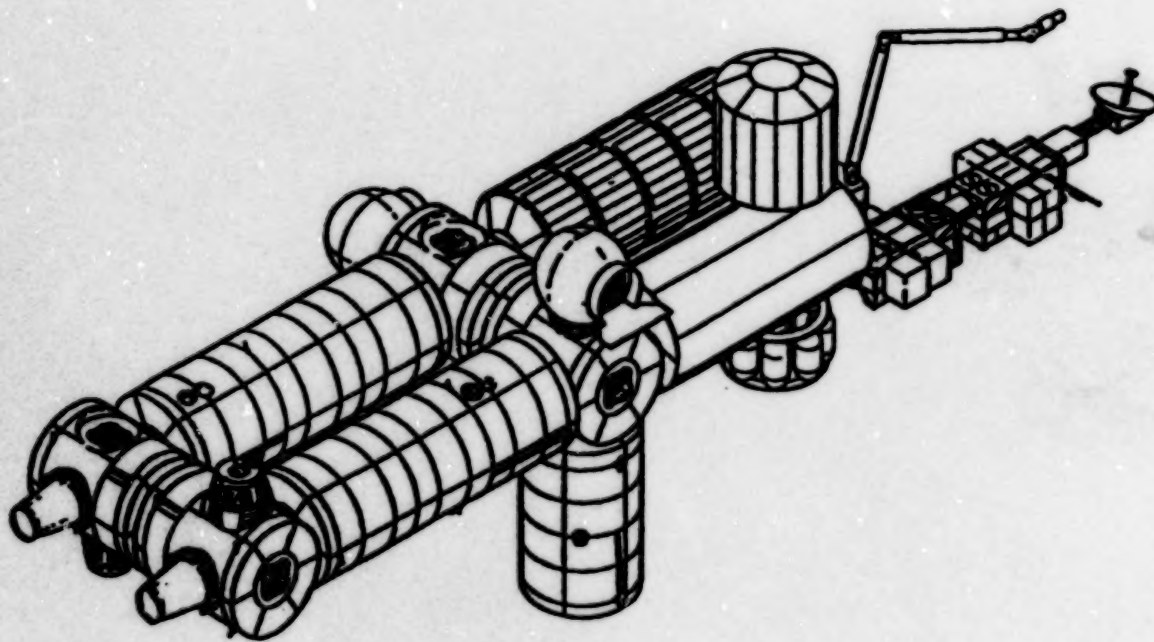


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Microbiology on Space Station *Freedom*



*Report of a panel discussion held at
the Lunar and Planetary Institute
Houston, Texas
November 6-8, 1989*

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Microbiology on Space Station *Freedom*

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1. PREFACE

Microorganisms are integral constituents of the environment and are primarily responsible for the recycling of biomass in nature. With their simple structure and dynamic physiology, microbes can adapt rapidly to environmental changes. They are capable of growing on almost every natural and synthetic material that attracts moisture and organic debris. Some microorganisms may produce environmental pollutants as metabolic byproducts; for example, acetaldehyde and isooctanol are produced by some fungi when colonizing polyurethane and polyvinylchloride, respectively.

Microorganisms affect human life in space in many ways, including risks related to in-flight infection or allergy as well as possible damage to the spacecraft itself through system fouling and biodeterioration.

Considering the number of known microbial species, only a relatively small number of microorganisms are known to cause serious diseases in healthy humans. The list of potential pathogens becomes nearly endless in compromised individuals. Recent studies suggest that microgravity, cosmic radiation, anxiety, and other stresses related to space travel may affect the immune system adversely. It is well known that even so-called "harmless" environmental or endogenous microbes may cause fatal infections in compromised hosts. It is, therefore, often impossible to delineate "harmless" microorganisms from those traditionally recognized as etiologic agents of disease. Thus, even innocuous constituents of the environmental and the endogenous flora could threaten health during long duration space missions.

Space Station *Freedom* will have a closed environmental system. Continual habitation, periodic crew exchange, docking of resupply vehicles, biological experiments, and the presence of experimental plants and animals on board for life-science study will contribute to its environmental microbial load. Because the capacity of the environmental control system to remove microorganisms is finite, many microorganisms will find niches in the interior of the spacecraft. The nature of the substrates and the effects of microgravity may induce changes in their metabolic activities through environmental selection, resulting in changes to their virulence and sensitivity to disinfectants and antibiotics.

A carefully designed program aimed at understanding the significance of the microbes present in the space station environment is of paramount importance. The objectives of this conference were to review, validate, and recommend revisions to the microbial acceptability standards proposed for air, water, and internal surfaces on board *Freedom*. In conjunction with the review of these standards, the advisory panel also evaluated the proposed microbiological capabilities and monitoring plan, and discussed disinfection procedures, waste management, and other clinical and crew issues.

2. EXECUTIVE SUMMARY

Microorganisms have a profound effect on the health and well-being of all inhabitants of Earth. The severe morbidity and mortality associated with epidemics and opportunistic infections in individuals are well documented. Most opportunistic infections are caused by either members of an individual's normal flora, microorganisms of environmental origin, or a combination of both. In a closed system such as Space Station *Freedom*, the potential morbidity and subsequent mortality resulting from microorganisms is very difficult to estimate; however, the experiences of the Soviet space program demonstrate that morbidity resulting from infection is not a theoretical issue. Microorganisms may elicit allergic reactions as well as infectious disease, both of which contribute to decrements in crew health and productivity. In addition to microorganisms brought to the *Freedom* environment by each crewmember during their tours of duty, it must be assumed that a resident microbial population will be established, and that this population will change over the life span of the station. As the station evolves, each crewmember will be at risk of infection or allergic reaction caused by the changing microbe populations originating from other crewmembers and from the station environment. In addition to crew health, the importance of microbes as agents of biodeterioration and potential harm to the station itself has not been adequately addressed.

This conference was convened for the purpose of reviewing NASA's plans for microbiology on Space Station *Freedom*. In the opinion of the advisory panel, ensuring the health of *Freedom* crews requires the establishment of a well-defined research effort to determine the potential effect of microorganisms on the crewmembers and on the physical environment of the space station. While the panel endorsed NASA's overall plan, they made several recommendations to address potential weaknesses in the plan.

The panel strongly recommended that the questions considered at this conference be answered by a consolidated, goal-oriented research program. There are very few data addressing the fundamental question of how microgravity influences microbial function. To answer this question, the panel advised using Earth-based microbial models, with subsequent validation by in-flight Shuttle data. The need for in-flight data, as well as reliable data collected immediately before launch and immediately after landing, was emphasized repeatedly throughout the conference.

The scope of NASA's research effort must accommodate the fact that as crews change and the station ages, specific environmental pressures will force microorganisms to respond to these stimuli by adapting to them. Crew health problems will parallel interactions between the crew's immune system and their resident flora and microbes in the physical environment of the station. The panel therefore stated that any standards formulated for *Freedom* must be viewed as dynamic rather than static, because they will require revision during the evolution of the station from assembly through habitation. The panel also advised that failure to implement such a research program may subject the crewmembers to unnecessary health risks.

Other specific suggestions as to appropriate standards, sampling techniques, and sampling frequencies can be found in Section 5 of this report. In conclusion, the panel recognized that NASA has a unique opportunity to assume a greater leadership role by actively supporting and guiding the necessary research efforts.

3. BACKGROUND INFORMATION

3.1 Space Station *Freedom*

Space Station *Freedom* (SSF), a major international development effort, will consist of four modules, four nodes, and several attached components. The two U.S. modules are each 44 feet long and 14 feet in diameter; one will function as a multipurpose laboratory area for conducting experiments in materials and life sciences, and the second will serve as the habitation area for the crewmembers. These two modules will be connected by four nodes, the precise dimensions of which are yet to be determined. Other components of *Freedom* will include a hyperbaric chamber, two international modules (the National Aerospace Development Agency of Japan's Japanese Experimental Module and the European Space Agency's *Columbus*), and logistics modules for transporting cargo and waste products to Earth.

It has been estimated that assembling the space station will require between 21 and 23 shuttle flights of varying duration, with frequent extravehicular activities (EVAs). During initial construction and assembly, crews will live in both the Orbiter and *Freedom*. As construction nears completion, a crew of four will live full-time on board *Freedom*; additional assigned crewmembers will bring the final crew to 8. Length of missions will increase incrementally from 30 days to 90 days, and then to a final maximum duration of 180 days. The first element launch for the project is scheduled for early 1995. The first manned mission is planned for mid 1997; the assembly process is anticipated to be complete in late 1998. The total life span of *Freedom* is expected to be 20 to 30 years.

The major health care issues that must be considered during the entire assembly process, from beginning to completion, include the physical stresses of microgravity, frequent EVAs during the construction stage, increased exposure to radiation, living in the closed environment with recycled air and water, and limited accessibility owing to the long turn-around time for a rescue mission. Issues of waste management and storage are also of concern, particularly after experimental plants and animals are brought on board.

Environmental and clinical microbiology capability on *Freedom* resides in two subsystems of the Crew Health Care System (CHeCS) (Table 1). The principal goal of the CHeCS is to protect the crew's health and maximize their operational efficiency. CHeCS is designed to provide medical diagnosis and treatment for critical and noncritical levels of care; to monitor the spacecraft environment; and to maintain the crew's physical conditioning during the physiological stresses of living in microgravity. Its three subsystems, the Health Maintenance Facility, the Environmental Health Subsystem, and the Exercise Countermeasures Facility, are described briefly below.

TABLE 1. CREW HEALTH CARE SYSTEM (CHeCS)

Health Maintenance Facility
* Medical operations support
Environmental Health Subsystem
* Environmental monitoring
Exercise Countermeasures Facility
* Physical conditioning equipment

The Health Maintenance Facility is designed to function as a remote medical facility. Crewmembers will have access to an extensive medical database, a hyperbaric chamber for use as necessary, and the capability of providing critical care life support for up to 45 days. The Environmental Health Subsystem monitors the station's environment, providing a means of detecting environmental hazards that can adversely affect the crew. The third subsystem, the Exercise Countermeasures Facility, contains several specialized computer-controlled exercise devices designed to counteract or minimize the negative physical effects of deconditioning.

The Environmental Control and Life Support System (ECLSS) will be designed and built to meet the medical requirements established by the Environmental Health Subsystem of CHeCS. The ECLSS consists of 7 subsystems (Table 2); the latter four subsystems, Waste Management, Atmosphere Control and Supply, Atmosphere Revitalization, and Water Recovery Management, are of particular interest at this conference.

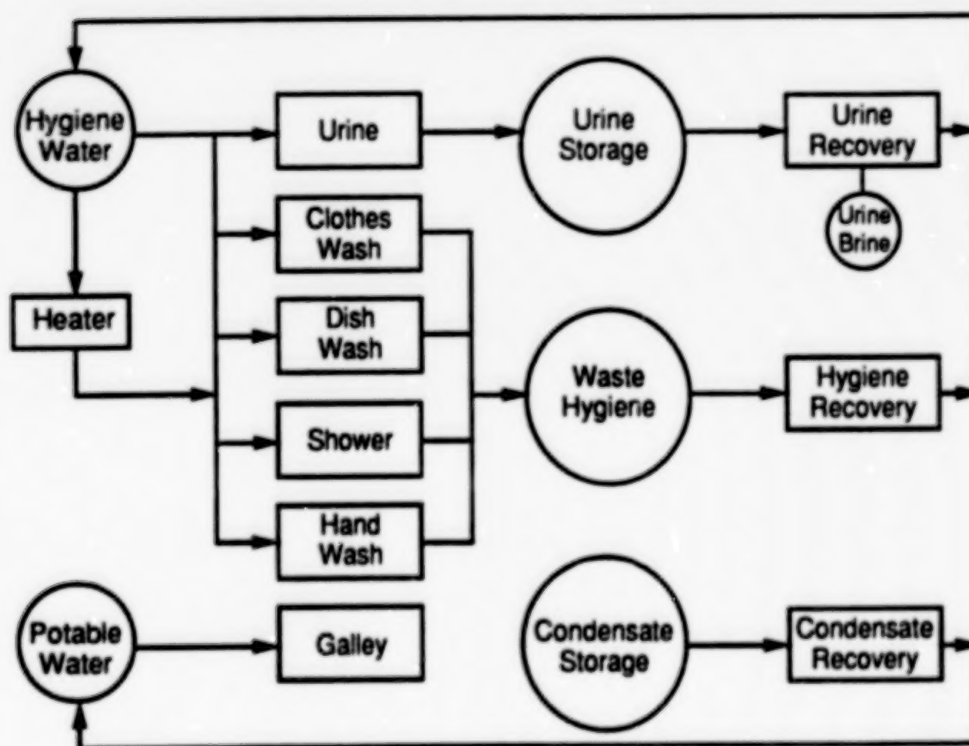
TABLE 2. ENVIRONMENTAL CONTROL & LIFE SUPPORT SYSTEM (ECLSS)

Extravehicular Activity Support (EVAs)
<ul style="list-style-type: none"> * Extravehicular servicing subsystem fluid supply and return * Airlock and hyperbaric chamber support
Temperature and Humidity Control (THC)
<ul style="list-style-type: none"> * Air temperature and humidity control * Ventilation * Equipment air cooling * Thermally conditioned storage
Fire Detection and Suppression (FDS)
Waste Management (WM)
<ul style="list-style-type: none"> * Return waste storage * Fecal waste processing
Atmosphere Control and Supply (ACS)
<ul style="list-style-type: none"> * Oxygen and nitrogen storage and distribution * Ventilation and relief * Oxygen and nitrogen pressure control
Atmosphere Revitalization (AR)
<ul style="list-style-type: none"> * Carbon dioxide reduction and removal * Oxygen generation
Water Recovery and Management (WRM)
<ul style="list-style-type: none"> * Urine processing * Hygiene water processing * Potable water processing * Water storage and distribution * Water thermal conditioning

Current requirements for volatile organic contaminants in respirable air are based upon 7-day spacecraft maximum acceptability concentrations; these values will be revised to reflect exposure periods of 90 days or more. Temperature and humidity control systems will be duplicated in order to provide redundancy as a safety feature. Air will be filtered with HEPA filters, which will be cleaned periodically by vacuuming their surfaces. Ventilation design includes atmospheric control and supply, and atmospheric revitalization. The latter includes schemes for carbon dioxide removal and control of trace contaminants.

Water Systems. Space Station *Freedom* will have two water systems, one that provides potable water and one that provides hygiene water (Figure 1). Each system will be duplicated so that the Habitation and Laboratory Modules will each have their own supply of both types of water. Water from these systems will be supplied to the two international modules as well. The microbial standard proposed for both water systems is 1 colony-forming unit (CFU) per 100 ml. Potable water will originate from condensate and carbon dioxide reduction, whereas hygiene water will be obtained from waste water originating from the galley, showers, and urine recycling. Hygiene water will not be recycled for use as potable water. Disinfection of both water systems will be accomplished chemically with iodine and physically with heat. An iodine concentration will be maintained between 1 and 4 ppm in the water systems by an iodinated exchange resin similar to that in the current Orbiter system. The two water systems will be static, with water from the processor units being distributed at 30 to 45 psia through either stainless steel, or possibly titanium pipes. Backflow will be prevented by maintaining positive pressure in the system. Check valves will also be installed if necessary.

FIGURE 1. Space Station Freedom Water Systems



In the potable water process, condensate water is collected, filtered, and then heated for 20 minutes at 250-270° F. The water exits through a regenerative heat exchanger and enters a multifiltration unit, where sorbent beds remove contaminants and maintain iodine level. After water quality is assessed by a process-control water-quality monitor, the water is pumped to a set of storage tanks. While the first tank is being filled, water in the second tank is held pending results of microbial testing, water in the third tank serves as a back-up, and water from the fourth tank is in use. Each 165-lb tank holds enough water for a crew of eight for two days.

In the hygiene water process, water is collected, filtered, and heated for 20 minutes at 250° F. The water exits through a regenerative heat exchanger and blends with reject water (brine) from the reverse-osmosis membrane module. The water mixture accumulates in a holding tank, passes through an ultrafiltration (UF) module, and proceeds on to the spiral-wound reverse osmosis membrane module, which removes inorganic and organic components. At this point, depending upon conductivity, the brine is either mixed with fresh feed or shunted to the urine processor. From the holding tank, the second-stage permeate enters posttreatment sorbent beds, where it is "polished" by a set of beds similar to those used in the potable water processor. Water quality measurements are performed and then the water is stored briefly in a preprocessing storage tank. Hygiene water will be processed continuously at a rate of 400-500 lbs per day. Urine will be processed separately. The processed urine is evaluated at the end of the processing loop and either sent to the hygiene water system or reprocessed through the urine system again. Wet solid wastes will not be recycled.

All water-recovery processors will be monitored for pH, iodine concentration (by ultraviolet absorption), conductivity, and total organic carbons (by infrared detection) by the Water Quality Control Monitors (WQCM). Gases present will be vented to trace contaminant control. If the water quality fails, the water stream is diverted back to the preprocessing storage tank and then reprocessed. The WQCMs will also monitor and analyze the potable and hygiene water systems at the storage tanks. The monitors will operate continuously and evaluate samples every 24 hours. Components to be analyzed include conductivity, pH, turbidity, color, ammonia, biocide level, specific ions, inorganic constituents, total organic carbons (TOCs), uncharacterized organic carbons (UTOCs), organic acids, complete organic constituents, total bacteria, dissolved gas, and free gas.

3.2 Some Physiological Effects of Space Flight

What effects will space flight have upon organisms? Can medical problems be predicted and prevented during space flight? Most of the data gathered to date in attempts to answer these fundamental questions have been obtained from Skylab, and a few 4- to 7-day Shuttle flights. Although the Soviet space station *Mir* has been manned continuously for periods of more than one year, *Mir* is not equipped to conduct routine biomedical studies.

One common "side effect" of space flight is space motion sickness. During the first few days of flight, approximately 75% of the crewmembers experience some symptoms of space motion sickness. The degree of illness varies depending upon the position and movements of the crewmembers, particularly movements of the head. Theories regarding the cause of space motion sickness have included sensory conflict, sensory-vestibular overload, and body fluid shifting; unfortunately, no predictors of who will experience space motion sickness have yet been defined.

Microgravity-induced shifting of body fluids toward the head, while probably not a cause of space motion sickness, has been shown to affect the functioning of the cardiovascular system. Cardiovascular investigators at JSC have developed a "stand

test" as a means of quantifying cardiovascular deconditioning, during which the subjects' heart rate and blood pressure are monitored during sudden changes in body position. One fairly successful technique developed for counteracting the cardiovascular effects of fluid shifting during space flight involves ingesting isotonic saline immediately before landing. "Saline loading" has been shown to affect the vascular space and musculature of blood vessels. Other techniques that are being explored include application of negative pressure to the lower body and the use of exercise training protocols.

Some of the other important physiological effects of space flight include an unexplained calcium loss, which leads to bone demineralization, and bone loss in the spine. Muscle mass and muscle strength are also lost, with roughly twice the strength being lost compared to the mass. Serum cortisol levels increase and then decrease proportionally with the length of time the crewmember spends in microgravity. Red blood cell mass decreases by approximately 15% within one to two months. The immunoglobulins increase in amount and then diminish. Mitogenic response of lymphocytes is decreased.

Infection is a documented problem, particularly urinary tract infections. The incidence of urinary tract infections in Apollo astronauts has resulted in a preflight quarantine policy. Two Soviet cosmonauts returned to Earth before their scheduled return because of infections that could not be treated in space. It is not clear whether these infections involved the urinary tract. Performance and behavior changes associated with isolation and confinement, particularly on long-duration flights, will affect crew compatibility. These and other physiological effects of space flight must be evaluated in order to ensure the health and well-being of the crew.

3.3 Microbiological Issues

While specimens for microbial analysis are collected routinely before and after Space Shuttle flights, opportunities for in-flight sampling have been limited. Sampling sites and materials have included air, internal surfaces, water, and food. Internal surfaces sampled include areas in the flight deck, the middeck, and Spacelab. According to current protocols, air and internal-surface samples are collected at launch minus 30 days, launch minus one day, and on the day of landing. The crew quarters are sampled 10 days before launch, and water on the Shuttle is sampled 20 days and 3 days before launch, and 5 days after landing, although concern has been raised that sampling water this late after landing is particularly misleading. Samples from the Shuttle's food lots are also sampled.

Air samples are taken using a centrifugal air sampler, which uses agar impaction as the collection principle. Different agar media are used in the sample strips to culture bacteria and fungi. One strip is intended to culture bacteria, whereas the second strip is used to monitor fungi. Air samples obtained from the Orbiter flight deck and middeck typically contained fungi such as *Aspergillus* and *Penicillium* spp., as well as bacteria like *Bacillus* and *Micrococcus* spp. It has been noted that *Staphylococcus* spp., normally associated with skin, have been recovered in large numbers from surfaces, but not from air samples.

In-flight sampling took place during STS-51B (Spacelab 3) because of microbial concerns involving the operation of the Research Animal Holding Facility (RAHF) in the Spacelab. Air samples, RAHF surface samples, and samples from crewmembers' throats and hands were taken during the mission. Numbers of airborne fungi and bacteria in the Spacelab tended to increase during this flight, to a maximum of 200 CFU/m³. Surface samples from the RAHF showed a predominance of *Staphylococcus* spp.; the crewmembers who changed the waste trays harbored *Staphylococcus* and *Streptococcus* spp.

Under the NASA Outreach Program, investigators at the University of Alabama at Huntsville have evaluated a number of potential monitoring methodologies for use on board *Freedom*. Such technologies must be capable of functioning in microgravity, manage multiple sample types, provide analytical data within 30 minutes to 6 hours, and fall within stringent power, weight, and volume constraints. Current methods with potential applications on board *Freedom* include particulate detection, culture technology, and recognition of indicator microorganisms. Some conventional methods for determining numbers of living microorganisms in ground-based models include membrane filtration, epifluorescent microscopy, direct viable counts, and microcolony enumeration either by direct microscopy or by using fluorescent indicators.

The aforementioned study evaluated 29 potential methods for "technological maturity" using weighted factors for various engineering requirements and feasibility criteria. Of these 29 methods, 6 fulfilled the following criteria: requires minimal modification to existing equipment; near real-time; nondestructive to living cells; noninvasive; requires minimal crew time; can potentially identify organisms; and possesses discriminatory capability. Technology is not available for detecting or estimating the amount of volatile or microbial products in real time.

3.4 Summary: JSC Microbiological Plan for Space Station *Freedom*

Plans for microbiology on board *Freedom* include analysis of clinical and environmental samples in order to maintain the health, safety, and productivity of the crew. Thus, the microbiology plan falls within two subsystems of the Crew Health Care System organization (Table 1). Clinical information will consist of microbial identification and determination of susceptibility to antimicrobial agents, whereas environmental data will be used to monitor microbes in air, in water, and on internal surfaces. The microbiological plan for *Freedom* must consider constraints involving limitation of equipment weight, power and volume, the amount of crew time and the crew's preference for completely automated systems, the amount of waste generated, maintenance requirements, shelf life of system components, and the reliability and validity of the data generated. The importance of having in-flight microbiological capability has been highlighted by the early termination of two Soviet flights due to infections during flight. It is clear that the lack of in-flight data, and the limitations on collecting "clean" preflight and postflight samples has resulted in a database that is insufficient to allow predictions of microbial behavior during extended missions.

Vitek's AutoMicrobic System II (AMS-II) has been designed for use in flight as a clinical microbiology assessment tool. Current requirements for clinical microbiology, as defined by the Health Maintenance Facility, include isolation and identification of pathogenic bacteria, fungi, and parasites from body fluids or tissues (blood, urine, feces, cerebrospinal fluid, wounds, skin, eye, and vagina) (Fig. 2); determination of the antimicrobial susceptibility of recovered pathogens; and adequate containment of microbiological specimens. The AMS-II will also be used to support the identification component of the environmental microbiology plan (Fig. 3).

FIGURE 2. Microbiological Assessment on Space Station (Clinical)

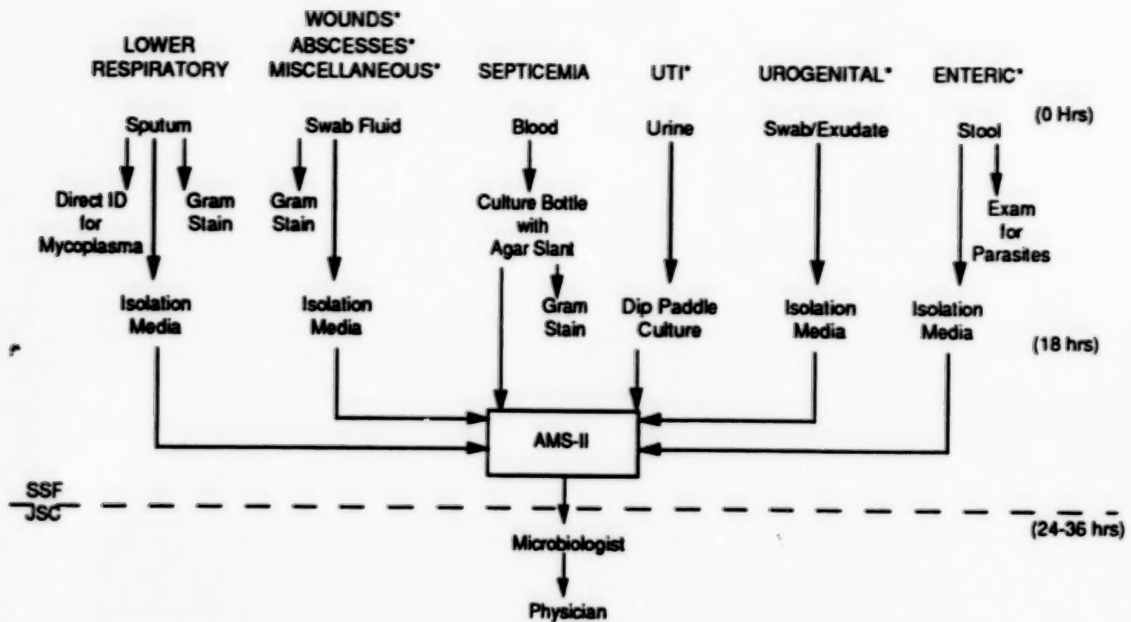
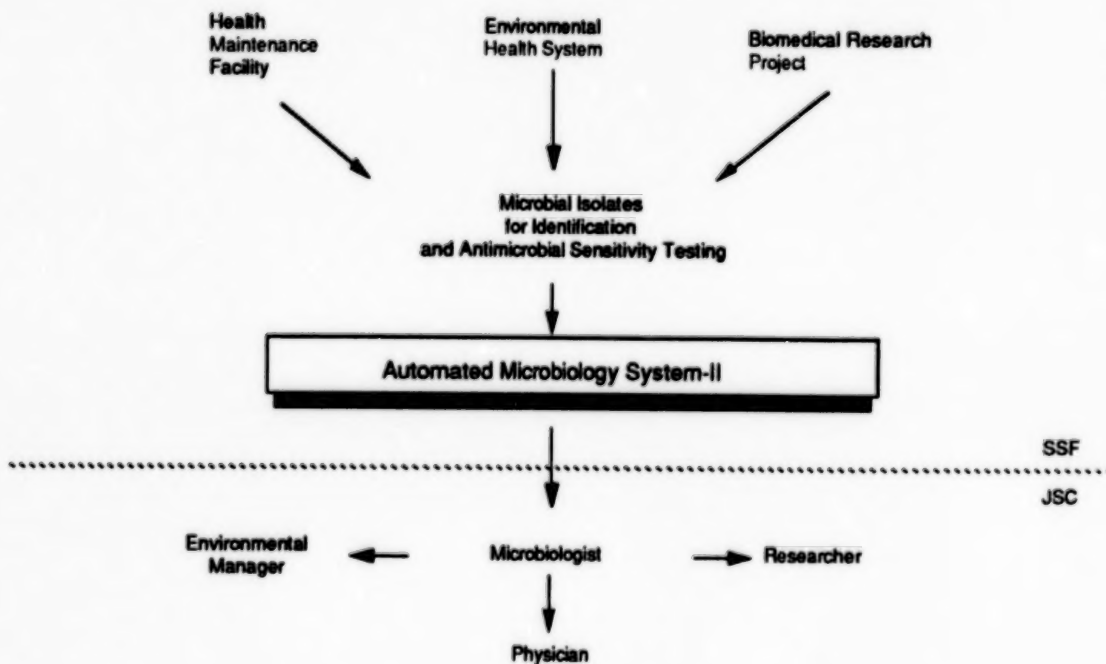


FIGURE 3. Microbiological Assessment on Space Station (Environmental)



The environmental microbiology plan includes microbial monitoring of air, internal surfaces, and water. Data obtained from most samples will be archived and transmitted to Earth for analysis. A summary of the current environmental standards and monitoring plans is presented in Table 4. Because current methodologies have not been designed for use in microgravity, a significant research effort will be required to develop the equipment needed for the monitoring protocols.

TABLE 4. PROPOSED ENVIRONMENTAL MICROBIOLOGY STANDARDS

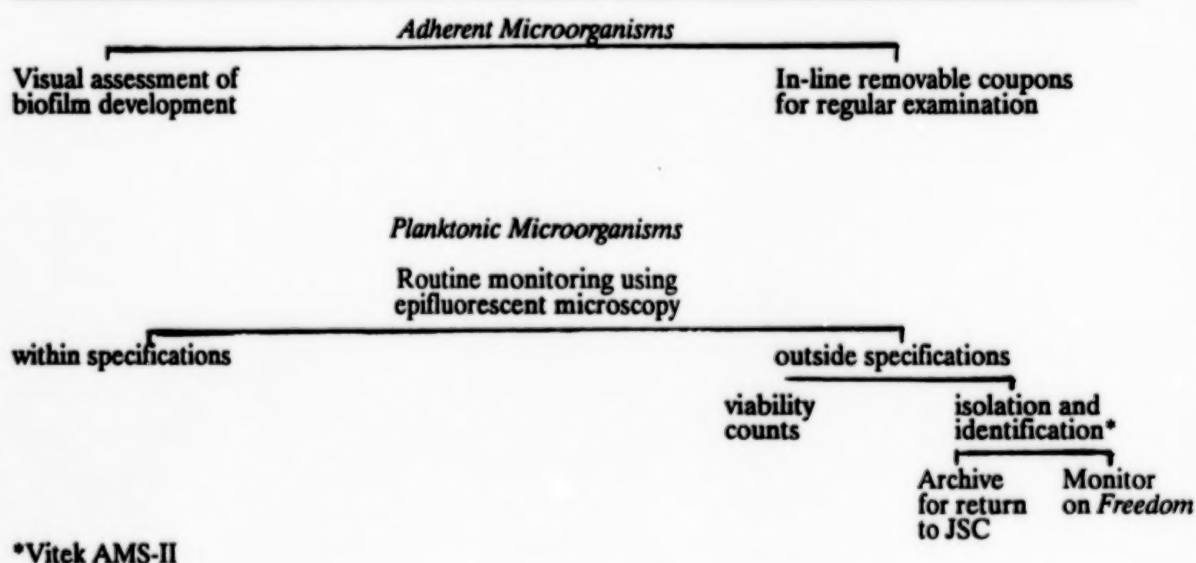
	<i>Air</i>	
Acceptability limits	500 CFU/M ³ *	
Instrument	Reuter Centrifugal Air Sampler	
Sampling frequency	Weekly	
	<i>Internal Surfaces</i>	
Acceptability limits	Undefined	
Instrument/Method	Swab, culture, or RODAC plates	
Monitoring frequency	As required	
	<i>Water</i>	
Acceptability limits	1 CFU/100 ml**	
Instrument/Method	Filtration/culture; epifluorescent microscopy	
Frequency	Each lot of reclaimed and recycled water	

*bacteria and fungi

**1 CFU, bacteria or fungi per 100 ml; 1 PFU, viruses per 100 ml

Water monitoring strategies for the potable and hygiene water systems will address both planktonic (free-floating) and adherent microorganisms. Because these microorganisms occupy different niches in the two water systems, different strategies to monitor them will be required (Fig. 4). As *Freedom* ages, the water quality requirements may change, especially as biofilms develop.

FIGURE 4. STRATEGIES FOR MONITORING WATER



Epifluorescent microscopy is the current method of choice for the routine monitoring of planktonic microorganisms. Drawbacks to this approach include the need for large volumes of water, extensive experience, and the toxicity of acridine orange, the stain proposed for direct microbial counts in water. Other techniques and stains are probably too labor-intensive because they require skillful handling. Techniques for determining viability counts using membrane filter culture or dehydrated media must be adapted to 0-g; for example, pouring agar plates is not feasible in microgravity. In addition, the amount of storage space will be restricted.

Protocols for monitoring adherent microorganisms occurring as biofilms have not yet been developed. Remediation, assessment, and identification are problems that need to be resolved. Visual assessment of biofilm development, in-line removable filters, and viable-count techniques are possible approaches to be incorporated into strategies for monitoring adherent microorganisms.

The conference panel was charged with reviewing and recommending revisions to the proposed standards for the microbiology program on board Space Station *Freedom* (Table 4), with particular attention to the following series of questions:

Air

- Is the present standard of 500 CFU/M³ an appropriate limit for air samples?
- Are acceptability limits for specific groups of airborne microorganisms necessary?
- How often should air be sampled?

Internal Surfaces

- Is surface sampling necessary?
- How often should surfaces be sampled?
- What are acceptable limits for surface samples?

Water

- How often should water be sampled?
- Is the present standard of 1 CFU/100 ml an appropriate limit for water samples?
- Which technologies for inflight verification of water sampling are appropriate?
- Are acceptability limits for specific groups of water-borne microorganisms necessary?

In summary, a number of problems specific to the space station environment will need to be overcome. In general, activities require greater manipulation and effort in microgravity than on Earth. The basic biology of the microorganisms encountered as contaminants may differ significantly from those organisms brought initially to *Freedom*. Factors like radiation, iodination, and oligotrophic (low nutrient) conditions may provide significant selective pressures. Because two separate water systems must be monitored, special problems associated with each may exist. Finally, the microbiological experience of the crewmembers may be minimal. Monitoring strategies must consider these aspects for the development of a sound program.

4. PANEL DISCUSSIONS

4.1 Monitoring Considerations

It is clear that criteria must be developed for monitoring, isolating, and identifying microorganisms present in the space station environment. The selection of sampling techniques will have a major effect on determining the appropriate standards. Determining the actual numbers of microorganisms in air, water, and on internal surfaces will be important, because comparisons of these numbers, as well as the taxa that are isolated, will permit the crew to detect microbial population changes. Specific indicator organisms should be selected, to include the microorganisms associated with biodeterioration and known potential pathogens. A series of standards that parallel the evolution of the station will be necessary for several reasons: The crew's immunologic function may change with increased time spent in microgravity; crews will be exchanged; and various selective pressures on *Freedom* will affect the microbial population on board. The entire process must be looked upon as being dynamic rather than static. Standards for acceptability will be affected by future needs and technological advancements. Monitoring protocols must be developed that correlate with the developmental stages of *Freedom*, from the initial construction period to the final functioning space station. Flexibility in system design is important because it will allow for technological upgrades. It will be paramount to understand the microbial dynamics of a closed system that operates in a microgravity environment.

4.1.1 Internal Surface Monitoring

The question was posed whether there are differences between the microbiology of air and internal surfaces in microgravity. The answer is probably yes, because internal surfaces will become contaminated by specific microorganisms associated with the movements of the crew. Individuals will tend to contact the same general areas as they move about in the station, thereby establishing a localized microbial flora at the contact sites. Selection of sampling sites should correlate with individuals' routes of travel and their work-flow patterns. Some areas, those touched frequently by the crew, should be considered at risk for surface contamination. These sites will correlate with personal habits, movements and work tasks, and may change significantly with the introduction of new crews and assigned work tasks.

Other surfaces of special importance will include ducts, condensation areas, and areas exhibiting visible growth. Wet surfaces, which may not always be visible, represent special potential problems. Hidden sites such as condenser coils may serve as localized niches where microorganisms such as fungi can proliferate. If at all possible, these sites should be cultured routinely for fungi. The panel recommended that access ports to such potential problem areas be provided for sampling. If growth were to occur in these unmonitored sites, microorganisms could potentially affect the baseline microbial population recovered in air samples in number, in taxa, or both.

The composition of different surfaces such as space suits, HEPA filters, fabrics, plastics, metals, glues, and rubbers may provide different substrates that can support the growth of different microorganisms. People may actually represent the most important surfaces for supporting microbial growth and shedding of microorganisms into the environment.

It is important to realize that the microbial burden on internal surfaces will not correlate with data from air samples. While surfaces will provide sites for microbial growth, air will act as a distribution vehicle for aerosolized organisms. These and similar considerations will be related to the surfaces selected for sampling, the sampling protocols, sample size and frequency of sampling, and the standards established to alert the crew that changes in the numbers of microorganisms or microbial taxa represent a potential or real problem that must be addressed promptly.

Sampling methods should be formulated for fungi, bacteria, and certain parasites but not viruses, since practical approaches for monitoring viruses and other organisms such as amoebae that could be present on *Freedom* do not exist at this time. The panel judged swabs to be an acceptable, simple technique for surface sampling; where culturing is appropriate, swabs could be inoculated subsequently to media for recovery of microorganisms. The use of acetate films or strip tape for fungal collection and identification by direct microscopy is a preferred approach. Fungi need not be cultured in all instances for identification. Ideally, the amount of isolation and identification work conducted on *Freedom*, particularly with fungi, should be kept to a minimum in order to reduce the potential of biohazards. Microscopic examination of specimens for fungi can be conducted on Earth via telemicroscopy. The composition of the microbial populations on board *Freedom* cannot be predicted at this time; however, it should remain relatively constant over the life of the station barring accidents, contamination, or infections. Because fungi produce airborne propagules, any amount of fungal growth in a closed system must be discouraged.

The change in number or type of microorganisms is more important than the actual numbers because this reflects the dynamics of population changes. In addition to monitoring for the environmental health aspects of crew safety, these data may be extremely important as a means to monitor for biodeterioration. The frequency of sampling should be correlated with the general housekeeping protocols for *Freedom*, because these will help determine the microbial load in the environment at a given time. The frequency and selection of sites for sampling will be significantly influenced by the routine cleaning schedules.

4.1.2 Air Monitoring

According to current plans, air in the space station environment will be passed through HEPA filters on the inlet side. Even though cabin air will be filtered, food particles and other residues are expected to be present in the air, and may become associated with internal surfaces. These particles can serve as nutrient substrates for microbial growth. Playing with food, as reported to occur in the Space Shuttle Program, should be discouraged. Free water in the air and associated with internal surfaces may pose additional problems. The HEPA filters will be replaced either every 90 days, or if the pressure across the filters drops. The panel noted that if the station air runs continuously, then changing the filters could contaminate the entire atmosphere. The panel recommended the addition of HEPA filters on the air outlet side as well as the air inlet side.

The panel recommended that sampling of air and surfaces be done wherever water condenses. Locations to be sampled will depend upon the atmospheric supply and its control mechanisms; sampling air vents for bacteria like *Legionella* should be

considered. Frequency of air sampling, like surface sampling, will depend upon crew activities, housekeeping chores, cleaning agents, and air distribution patterns. Although a firm recommendation cannot be made at this time, biweekly monitoring would be considered appropriate.

As to numerical standards, the panel felt that the proposed standard of 500 CFU/m³ is too low, and recommended that at least two acceptability standards be established instead, one for bacteria and another for fungi. These standards, whether numerical or taxonomic, should reflect the chosen method of data gathering and analysis. The Reuter centrifugal air sampler (RCS), for example, is not an efficient means of monitoring fungi, nor is rose bengal agar appropriate for their selective isolation. Air sampling methods and corresponding standards should be based upon numbers of propagules per unit of air sampled. For example, a standard acceptable for use of the RCS would be 500 CFU/m³, whereas 1500 CFU/m² would be realistic for a particulate sampler.

Routinely cultivating fungi from air samples will probably not be necessary; fungi can instead be trapped on microscope slides for microscopic examination. It should be noted that fungal-bacterial aggregates are more likely to occur. Cultivating mixed microbial populations of bacteria and fungi will require some microbiological skill. Unfortunately, crewmembers skilled in microbiological techniques may not be present. Of equal concern, the time necessary to spend on microbial isolation, isolate purification, and isolate identification procedures will most likely not be available. Like internal surfaces, the types of air environments to be sampled, the selection of sites, frequency of sampling, and methods to be used are important components of the monitoring plan and the standards to be followed.

4.1.3 Water Monitoring

For consistency, water standards should be the same for both the potable and hygiene water systems, and these standards should reflect the effectiveness of the two systems in keeping the water microbe-free. The panel felt that the number of bacteria present is a more meaningful criteria than the presence or absence of coliform bacteria. They judged that the proposed standard of 1 CFU/100 ml is unrealistic. To achieve a standard of 1 CFU/100 ml would be expensive and labor-intensive, if it could be done at all. A 1-ml unit was thought to be more appropriate because of the limited amount of water on *Freedom*. The panel recommended adopting the National Committee for Clinical Laboratory Standardization (NCCLS) standard of 10 CFU/ml, which was established for heat or filter-sterilized distilled water coming directly from a water tap. The presence of 10 CFU/ml can be monitored easily with the dipstick technique. This standard will probably have to be modified over time because of the eventual development of biofilms, which will significantly affect the numbers of organisms in the water systems. The particulates that slough off from biofilms within water systems can contain as many as 10⁴ to 10⁸ organisms/ml.

The current standard for viruses is 1 PFU/100 ml. While the panel believed this standard should be zero, it was acknowledged that methods to detect viruses in water are not readily available. In addition to standards for numbers of organisms, it was suggested that a limit of 10 ug/ml for microbial carbon products be established, since these products provide carbon sources for growth of organisms and biofilm formation. The issue of identifying specific taxa also must be addressed. It is believed that the

filters in the currently planned system will filter out obvious pathogens from the water, which places a greater emphasis upon identifying potential opportunistic pathogens. In any case, the detection level established must be well below potential infectious levels in order to ensure crew safety.

The selection of sampling techniques for use on *Freedom* must take into consideration a number of points. In order to provide meaningful data, water from the two water systems should be tested at the point of use, not in the tanks. The quality of the water entering the system is critical. The sampling method adopted should provide a rapid turn-around time for isolation and subsequent identification. Rapid methods often rely upon complex equipment that will probably exceed cost, volume, weight, and power constraints. Even though approaches like dipstick and filtration methods are not necessarily the fastest, they are more suited for *Freedom*. Sampling frequency must take into account the effectiveness of the water treatment process.

The design and implementation of the water treatment system is of paramount importance to the quality of the water produced. Experience with the Research Animal Holding Facility at Ames Research Center suggests that it is very difficult to keep condensate water free of microorganisms. Under the current system, water is moved rapidly from the raw form through the treatment process to the consumption state, because holding hygiene water for longer than 24 hours creates insurmountable storage problems. The water system should be processing continuously, since turning the systems on and off will lead to instability of treatment barriers and possible breakthrough of microorganisms that proliferate in treatment stages. Although heat is an effective disinfectant, dead spots in the heat-processing component of the system are cause for concern. The panel questioned the 20-minute 250°F treatment process, and strongly recommended instituting fail-safe mechanisms such as recirculation should the heating cycle or temperature fall below standard.

The treatment train design must include provisions for the eventual development of microbial resistance to disinfectants like chlorine and iodine. Studies using silver chloride resins at the ends of water-treatment systems have shown that microorganisms can develop resistance within weeks. Such data raise the question of whether iodinating the water at the end of the treatment process will be useful. Plans should be formulated for decontaminating the water systems in the event that they do become contaminated and the disinfectants are no longer effective.

Finally, much concern was expressed regarding biofilm development on *Freedom*. Organic and inorganic contaminants will form a nutrient base for biofilm development. The reduction of biofilms should not be attempted with either ozone or heat, since both processes degrade the biofilms into smaller compounds that provide a nutrient base for new biofilm formation.

The panel strongly recommended establishing the entire water reclamation system as a test bed at NASA and studying it for at least two years. Such a model could be challenged with potential microbial problems. Organisms to be considered in the evaluation of the system should include hepatitis A virus, *Cryptosporidium* spp., *Klebsiella pneumoniae*, *Escherichia coli* serotype 0157:H7, *Mycobacterium* spp., poliovirus, MS2, and parvovirus.

4.2 Prevention and Disinfection

As a means of reducing the numbers of microorganisms associated with surfaces, it was suggested that surface materials be impregnated with antimicrobial agents. However, because these agents will eventually leach from surfaces (off-gas) and enter the atmosphere, toxicological problems may arise. An alternative suggestion for sterilizing surfaces was ultraviolet light. However, this method has several disadvantages: it requires too much power, and cadmium and mercury are banned from use on board *Freedom*. Disinfectants like hydrogen peroxide may represent a simple solution to the disinfection issue. Energy should be devoted to evaluating possible candidate disinfectants. In general, the numbers of microorganisms can be greatly reduced if the relative humidity of the atmosphere were to be maintained below 50%.

The panel proposed establishing a ground-based space station mockup for use as a model for microbial study. Such a model could be useful in characterizing the resident flora, with and without crew habitation.

4.3 Containment and Waste Management

Since microorganisms in the space station environment as well as in clinical specimens are potential opportunistic pathogens, a biological safety cabinet is an absolute requirement for the microbiology monitoring program. The safety cabinet must be used when processing samples originating from air, water, internal surfaces and clinical specimens. Crewmembers must be especially careful when handling cultures in liquid media in microgravity. Extreme care must be used to ensure that fungi maintained in a containment facility are not released into the environment. It was recommended that incubators and waste disposal units interface directly with the biological safety cabinet to ensure that microorganisms do not escape. Because of the physical difficulties of working in microgravity, the panel recommended that the safety cabinet be at the P-3 level. Disinfection of work surfaces in the biological safety cabinet, as well as other surfaces, must be well planned. Because *Freedom* is a closed system, localized contamination incidents could become station-wide problems within an extremely short time. Disinfectants like hydrogen peroxide, chlorhexidine, or betadine were suggested to be used for wiping surfaces. Alternative disinfection processes, such as passing incubator components through disinfectants like paraformaldehyde, alcohol, chlorine, or quarternary ammonium compounds pose unacceptable toxicological or fire hazards. Using autoclaves is not practical because of excessive power consumption.

The air flow design for the biological safety cabinet has not yet been established. In addition, the method for evacuation of waste that is generated in the biological safety cabinet must be determined. McDonnell-Douglas is currently addressing these design concerns.

Much of the microbiology to be conducted on *Freedom* will rely upon microscopy. The panel felt that the microscope could be housed separately from the biological safety cabinet. Placement of the microscope in the biological safety cabinet would reduce the amount of available workspace. In addition, the microscope could easily become contaminated. It was suggested that a video camera should be used in place of the microscope eye pieces, with the eye pieces being available on board. Having telemicroscopic-telerobotic capability on board would reduce the crew time required to analyze mixed samples. Video or still images could be transmitted easily to a ground base, where they could be analyzed by experienced microbiologists. The location of the microscope requires further discussion.

4.4 Clinical and Crew Issues

Because microbial problems have been noted after brief extravehicular activities (EVAs), space suits should be monitored for contamination. This will be especially important during the early assembly stages of the Station because of the many EVAs that will be required. It is important to understand that space suits may develop their own microbial ecosystems through accumulation of body fluids and associated flora.

In order to understand microbial dynamics in a closed microgravitational system, it was suggested that baselines for normal crewmember flora be determined, and crewmembers monitored routinely thereafter. At this time, there are no standards for this type of data. Because it is possible that an immunocompromised state of some degree may develop during long space flights, baseline data on microbial flora, both clinical and environmental, will be necessary in order to protect the health of the crew as their susceptibility increases.

5. CONCLUSIONS AND RECOMMENDATIONS

A panel of distinguished scientists was charged with reviewing and recommending revisions to NASA - Johnson Space Center's microbiological plan for Space Station *Freedom*. The panel endorsed the overall plan, which is described in detail in Section 3.4, and offered the following conclusions and recommendations.

In order to protect crew health, safety, and productivity on board *Freedom*, the standards formulated for clinical and environmental microbial analysis must be viewed as dynamic rather than static. During the station's evolution from assembly to habitation and throughout its projected lifetime, these standards will require revision because of the significant microbial population changes that will likely occur. In addition, standards will also change as technologies for the detection of microbes improve.

In general, availability of sampling techniques and their application will have a profound effect on the establishment of the initial standards for the station. In order to effectively monitor microbial population dynamics, microbial numbers as well as taxa must be determined in air, on internal surfaces, and in water. It should be noted that when setting quantitative limits, all microorganisms cannot be treated alike. Specific indicator organisms should be selected, as well as microorganisms that must be excluded from the station. As an example, those microorganisms listed as Class 2 (National Institutes of Health - Centers for Disease Control Classification) (Table 5) should be viewed with great caution. The presence of Class 3, 4 or 5 microorganisms should be considered totally unacceptable.

The panel's specific observations and recommendations are listed below.

Surface Monitoring:

Surface sampling was agreed to be necessary, but can be limited to areas at high risk of contamination.

Sampling sites should include places that the crew physically touches, as well as areas in which water condenses, and areas of visible growth.

Selection of sampling sites should take into account the composition of the surfaces to be sampled, as each has the potential to serve as a substrate for different types of organisms.

The frequency with which surfaces should be sampled will be influenced significantly by housecleaning procedures on board *Freedom*, and cannot be established at this time.

The panel did not reach a consensus on acceptable limits for surface contamination, but recommended that changes in the numbers or types of microorganisms should be monitored rather than actual microbial counts, because changes reflect microbial population dynamics.

Surfaces can be sampled with swabs, with subsequent inoculation to media when necessary.

Isolation and identification, particularly of fungi, should be kept to a minimum in order to avoid station-wide contamination.

Air Monitoring:

An appropriate frequency for air sampling cannot be established without additional information on the filter system.

A second HEPA filter should be added on the air outlet side.

The method of changing air filters should be selected carefully. If the station's air system runs continuously, changing the filters could contaminate the entire atmosphere.

Air, like surfaces, should be monitored wherever water condenses.

Separate numerical standards should be established for bacteria and for fungi; 500 CFU / m³ may be too stringent for bacteria plus fungi.

Cultivation of fungi should be kept to an absolute minimum; trapping fungi on slides for microscopic analysis may be sufficient, particularly if downlinking is available for Earth-based analysis.

Water Monitoring:

Water standards should be the same for both the potable and the hygiene water systems.

Water should be tested at the faucet, not in the storage tanks.

Fail-safe mechanisms, such as water recirculation, should be instituted in the event of heating-cycle failure.

The current standard of 1 CFU / 100 ml for bacteria, yeasts, and moulds was judged too stringent and cannot be defended on the basis of crew health. The panel recommended adopting the National Committee for Clinical Laboratory Standardization standard of 10 CFU / ml, with the understanding that this standard will change over time as biofilms develop in the water supply.

A standard of 10 CFU/ml can be monitored easily during flight with dipstick technology.

While the standard for viruses and other enteric organisms should be zero, it is acknowledged that detection methods are not presently available.

Ten ug/ml of microbial carbon products should be introduced as a standard.

The capability of identifying specific taxa is required in order to identify potential opportunistic pathogens.

Sampling frequency must take into account the effectiveness of the treatment train, and cannot be determined at this time.

A water system test bed should be run on Earth for at least 2 years, and challenged microbially before the system is instituted in flight.

Prevention and Disinfection:

Candidate disinfectants should be evaluated for their potential toxicologic effects.

Microbial numbers can be reduced considerably if the relative humidity is maintained below 50%.

A ground-based space station mockup should be used for characterizing the microbial flora, both with and without crew habitation.

Containment and Waste Management:

A biological safety cabinet is an absolute requirement. Incubators and waste disposal units should be interfaced directly with the unit.

Surfaces should be wiped down with disinfectants such as hydrogen peroxide, chlorhexidine, or betadine.

Air flow in the cabinet, and evacuation of waste from it, must be determined.

The microscope should be located outside of the biological safety cabinet.

Clinical and Crew issues:

Space suits should be monitored regularly for contamination.

Baselines for normal crew flora should be established, and crewmembers monitored routinely afterward.

TABLE 5.

NATIONAL INSTITUTES OF HEALTH - CENTERS FOR DISEASE CONTROL
CLASSIFICATION OF MICROORGANISMS ON THE BASIS OF HAZARD

CLASS 2 AGENTS

Bacterial Agents

Acinetobacter calcoaceticus
Actinobacillus spp.
Actinomyces spp.
Arachnia propionica
Aeromonas hydrophila
Arizona hinshawii (all serotypes)
Bacillus anthracis
Bordetella spp.
Borrelia recurrentis
B. vincentii
Campylobacter fetus
C. jejuni
Chlamydia psittaci
Ch. trachomatis
Clostridium botulinum
Cl. chauvoei
Cl. haemolyticum
Cl. histolyticum
Cl. novyi
Cl. septicum
Cl. tetani
Corynebacterium diphtheriae
C. equi
C. haemolyticum
C. pseudotuberculosis
C. pyogenes
C. renale
Edwardsiella tarda
Erysipelothrix insidiosus
Escherichia coli
 *all enteropathogenic, enterotoxigenic, enteroinvasive, and strains bearing K1 antigen
Haemophilus ducreyi
H. influenzae
Klebsiella spp. (and serotypes)
Legionella pneumophila
Leptospira interrogans spp.
Listeria spp.
Moraxella spp.
Mycobacterium spp. (except Class 3)
Mycoplasma spp.
 (except *Mycoplasma mycoides* and *Mycoplasma agalactiae*) (Class 5)
Neisseria gonorrhoeae
N. meningitidis
Nocardia spp.
Pasteurella spp. (except Class 3)
Salmonella spp. (and serotypes)
Shigella spp. (and serotypes)
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pneumoniae
S. pyogenes
Treponema carateum
T. pallidum
T. pertenue
Vibria cholerae
V. parahaemolyticus
Yersinia enterocolitica

Fungal Agents

Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides brasiliensis

Parasitic Agents

Entamoeba histolytica
Leishmania spp. (sp)
Naegleria gruberi
N. fowleri
Schistosoma mansoni
Toxoplasma gondii
Toxocara canis
Trichinella spiralis
Trypanosoma cruzi

Viral, Rickettsial, and Chlamydial Agents

Adenoviruses (human, all types)
 Cache Valley Virus
 Coronaviruses
 Coxsackie A and B viruses
 Cytomegaloviruses
 Echoviruses (all types)
 Encephalomyocarditis virus (EMC)
 Flanders virus
 Hart Park virus
 Hepatitis-associated antigen material
 Herpesvirus-associated antigen material
 Herpesviruses (except *Herpesvirus simiae*) (Class 4)
 HTLV I/II
 HIV
 Influenza viruses (except A/PR8/34) (Class 1)
 Langat virus
 Measles virus
 Mumps virus
 Parainfluenza viruses (except virus 4, SF 4 strain) (Class 1)
 Polioviruses (all types, wild & attenuated)
 Poxviruses (except Alastrim, Smallpox, & Whitepox) (Class 5)
 (except Monkey pox) (Class 3 or 4)
 Rabies virus (except Rabies street virus) (Class 3 or 4)
 Reoviruses, all types
 Respiratory syncytial virus
 Rhinoviruses, all types
Rochalimaea insonii
 Rubella virus
 Simian viruses (except *Herpesvirus simiae* & Marburg virus) (Class 4)
 Sindbis virus
 Tensaw virus
 Turlock virus
 Vaccinia virus
 Varicella virus
 Vesicular stomatitis virus
 Yellow fever virus (17D vaccine strain)

TABLE 5, continued

CLASS 3 AGENTS

Bacterial Agents

Bartonella spp.
Brucella spp.
Francisella tularensis
Mycobacterium avium complex
M. bovis
M. tuberculosis
Pasteurella multocida type B
Pseudomonas mallei
P. pseudomallei
Yersinia pestis

Fungal Agents

Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var *duboisii*

Parasitic Agents

none

Viral, Rickettsial, and Chlamydial Agents

Arboviruses
 (except members of Classes 2 & 4)
Coxiella burnetii
Ehrlichia spp.
 Lymphocytic choriomeningitis virus
 (LMC)
 Monkey pox virus*
 Rabies street virus
Rickettsia spp. (except *R. ruminantium*)
 West Nile virus
 Semliki Forest virus
 Dengue virus**
 Yellow fever virus (wild)*

CLASS 4 AGENTS

Bacterial, Fungal, Parasitic Agents

none

Viral, Rickettsial, and Chlamydial Agents

Ebola fever virus
 Hemorrhagic fever agents:
 Crimean hemorrhagic fever
 Congo virus
 Junin virus
 Machupo virus
Herpesvirus simiae (Monkey B virus)
 Lassa fever virus (*Mastomys natalensis*)
 Marburg virus (*Cercopithecus* spp.)
 Monkey pox**
 Tick-borne encephalitis virus complex:
 Russian spring-summer encephalitis
 Kyasanur forest disease
 Omsk hemorrhagic fever
 Central European encephalitis viruses
 Venezuelan equine encephalitis virus
 (epidemic strains)**
 Yellow fever virus (wild)**

*when used in vitro

**when used for transmission or animal experiments

TABLE 5, continued
CLASS 5 AGENTS

AD2-SV40**
AD7-SV40*
Adenovirus*
African horse sickness virus
African swine fever virus
Alastrim
Avian Leukosis*
Besnoitia besnoiti
Borna disease virus
Bovine infectious petechial fever agent
Bovine Leukemia*
Bovine Papilloma*
Camelpox virus
CELO*
Dog Sarcoma*
EBV**
Ephemeral fever virus
FeLV**
FeSV**
Foot and mouth disease virus
Fowl plague virus
GaLV**
Goat pox virus
Guinea Pig Herpes*
Hamster Leukemia*
HTLV I/II*
Hog cholera virus
HV Ateles**
HV Saimiri**
Louping ill virus
Lucke (Frog)*
Lumpy skin disease virus

Marek's virus*
Mason-Pfizer Monkey Virus*
Mouse Mammary Tumor*
Murine Leukemia*
Murine Sarcoma*
Mycoplasma agalactiae
M. mycoides
Nairobi sheep disease virus
Newcastle disease virus (velogenic strains)
Polyoma*
Rat Leukemia*
Rat Mammary Tumor*
Rickettsia ruminantium (heart water)
Rift Valley fever virus
Rhinderpest virus
Rous Sarcoma*
Sheeppox virus
Shope Fibroma*
Shope Papilloma*
Smallpox
SSV-1**
SV-40*
Swine vesicular disease virus
Teschén disease virus
Theileria annulata
T. bovis
T. hirei
T. lawrencei
T. parva (East Coast Fever)
Trypanosoma evansi
Tr. vivax (nagana)
Vesicular exanthema virus
Wesselsbron disease virus
Whitepox
Yaba**
Zygonema

*low-risk oncogenic virus

**moderate-risk oncogenic virus

6. PANELIST PRESENTATIONS

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Biodegradation

Microorganisms, in addition to potentially causing opportunistic infections, may cause the biodeterioration of surfaces, particularly fabrics. Fabrics containing wool or cotton are often damaged by microbes. Antimicrobial compounds incorporated into fabrics, rubbers, plastics and other components of SSF may be required as a means of preventing or reducing biodeterioration of surfaces caused by microbes.

The numbers of microorganisms are more important than the taxa involved. An exception, of course, would be the unlikely presence of an established mould pathogen or pathogenic bacterium. Monitoring programs must emphasize the microbial load rather than the taxa of microorganisms. The microorganisms that are associated with biodegradation processes do not occur as individual components such as conidia, but rather consist of mycelial clumps that often contain bacteria. Any system used to monitor microorganisms on internal surfaces, in air, and in water must be sensitive to the importance of microorganisms capable of causing biodeterioration. Quantitative sampling will be an extremely important activity.

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Diseases Caused by Common Airborne Fungi

A broad spectrum of filamentous fungi can be potential opportunistic pathogens in the immunocompromised host. Most of these infections would result from inhaling conidia or spores by individuals that have compromised immune systems. Because a large number of these fungi (Tables 6 and 7) could be present in the SSF environment, it will be important to monitor the environment for their presence.

Most of the potentially dangerous opportunistic fungi will be exogenous, originating from items brought to the SSF from Earth. However, endogenous potential opportunistic pathogens such as *Candida albicans* can also cause serious infections.

Table 6. Known Agents of Hyalohyphomycosis

Genera and Species	Genera and Species
<i>Acremonium</i>	<i>P. marquandii</i> <i>P. variotii</i>
<i>A. alabamensis</i>	<i>Penicillium</i>
<i>A. curvulum</i>	<i>P. chrysogenum</i>
<i>A. falciforme</i>	<i>P. citrinum</i>
<i>A. kiliense</i>	<i>P. expansum</i>
<i>A. potronii</i>	<i>P. spinulosum</i>
<i>A. roseo-griseum</i>	
<i>Anxiopsis</i>	<i>Pseudallescheria</i>
<i>A. fulvescens</i>	<i>P. boydii</i>
<i>A. stereocaria</i>	
<i>Beauveria</i>	<i>Scedosporium</i>
<i>B. alba</i>	<i>S. inflatum</i>
<i>B. bassiana</i>	
<i>Coprinus</i>	<i>Schizophyllum</i>
<i>C. cinereus</i>	<i>S. commune</i>
<i>Cylindrocarpon</i>	<i>Scopulariopsis</i>
<i>C. lichenicola</i> (<i>C. tonkinense</i>)	<i>S. acremonium</i>
<i>C. vaginae</i>	<i>S. brevicaulus</i>
<i>Fusarium</i>	<i>Scytalidium</i>
<i>F. chlamydosporum</i>	<i>S. hyalinum</i>
<i>F. dimerum</i>	
<i>F. episphaeria</i>	<i>Tritirachium</i>
<i>F. moniliforme</i>	<i>T. oryzae</i>
<i>F. nivale</i>	
<i>F. oxysporum</i>	<i>Tubercularia</i>
<i>F. proliferatum</i>	<i>T. vulgaris</i>
<i>F. sacchari</i>	
<i>F. solani</i>	
<i>F. verticilloides</i>	
<i>Lecytophora</i>	<i>Volutella</i>
<i>L. hoffmannii</i>	<i>V. cinerescens</i>
<i>L. mutabilis</i>	
<i>Microascus</i>	
<i>M. cinereus</i>	
<i>Myriodontium</i>	
<i>M. kertinophilum</i>	
<i>Paecilomyces</i>	
<i>P. fumoso-roseus</i>	
<i>P. lilacinus</i>	

Table 7. Known Agents of Phaeohyphomycosis

Genera and Species	Genera and Species
<i>Alternaria</i>	<i>Curvularia</i>
<i>A. alternata</i>	<i>C. geniculata</i>
<i>A. chartarum</i>	<i>C. lunata</i>
<i>A. dianthicola</i>	<i>C. pallescens</i>
<i>A. infectoria</i>	<i>C. senegalensis</i>
<i>A. stemphyloides</i>	<i>C. verruculosa</i>
<i>A. tenuissima</i>	
<i>Anthopsis</i>	<i>Dissitimus</i>
<i>A. deltoidea</i>	<i>D. exedrus</i>
<i>Arinum</i>	<i>Drechslera</i>
<i>A. leporinum</i>	<i>D. biseptata</i>
<i>Aureobasidium</i>	<i>Exophiala</i>
<i>A. pullulans</i>	<i>E. castellanii</i>
<i>Bipolaris</i>	<i>E. jeanselmei</i>
<i>B. australiensis</i>	<i>E. monilae</i>
<i>B. hawaiiensis</i>	<i>E. pisciphila</i>
<i>B. spicifera</i>	<i>E. salmonis</i>
<i>Botryomyces</i>	<i>E. spinifera</i>
<i>B. caespitosus</i>	<i>Exserohilum</i>
<i>Chaetomium</i>	<i>E. longirostratum</i>
<i>C. funicola</i>	<i>E. mcginnisii</i>
<i>C. globosum</i>	<i>E. rostratum</i>
<i>Cladorrhinum</i>	<i>Fonsecaea</i>
<i>C. bulbiliosum</i>	<i>F. pedrosoi</i>
<i>Cladosporium</i>	<i>Lasodiplotia</i>
<i>C. cladosporioides</i>	<i>L. theobromae</i>
<i>C. devriesii</i>	<i>Moniliella</i>
<i>C. elatum</i>	<i>M. suaveolens</i>
<i>C. oxysporum</i>	<i>Mycocentrospora</i>
<i>C. sphaerospermum</i>	<i>M. acerina</i>
<i>Colletotrichum</i>	<i>Natrassia</i>
<i>C. dematium</i>	<i>N. mangiferae</i>
<i>Coniothyrium</i>	
<i>C. fuckelii</i>	

Table 7. Known Agents of Phaeohyphomycosis, continued

Genera and Species	Genera and Species
<i>Peyronellaea</i>	<i>Pyrenochaeta</i>
<i>P. glomerata</i>	<i>P. unguis-hominis</i>
<i>Phaeoannellomyces</i>	<i>Ramichloridium</i>
<i>P. elegans</i>	<i>R. schulzeri</i>
<i>P. wernickii</i>	
<i>Phaeosclera</i>	<i>Sarcinomyces</i>
<i>P. dermatioides</i>	<i>S. phaeomuriformis</i>
<i>Phaeotrichoconis</i>	<i>Scolecobasidium</i>
<i>P. crotalariae</i>	<i>S. gallopavum</i>
	<i>S. humicola</i>
	<i>S. tshawytscha</i>
<i>Phialemonium</i>	<i>Scytalidium</i>
<i>P. obovatum</i>	<i>S. lignicola</i>
<i>Phialophora</i>	<i>Stenella</i>
<i>P. bubakii</i>	<i>S. araguata</i>
<i>P. parasitica</i>	
<i>P. repens</i>	
<i>P. richardsiae</i>	
<i>P. verrucosa</i>	
<i>Pleurophoma</i>	<i>Taeniolella</i>
<i>P. pleurospora</i>	<i>T. stilbospora</i>
<i>Phoma</i>	<i>Tetraploa</i>
<i>P. cava</i>	<i>T. aristata</i>
<i>P. cruris-hominis</i>	
<i>P. eugyrena</i>	<i>Trichomarix</i>
<i>P. herbarum</i>	<i>T. invadens</i>
<i>P. hibernica</i>	
<i>P. minutella</i>	
<i>P. oculo-hominis</i>	<i>Ulocladium</i>
<i>Phyllosticta</i>	<i>U. chartarum</i>
<i>P. citricarpa</i>	
<i>P. species</i>	<i>Wangiella</i>
<i>Pseudomicrodochium</i>	<i>W. dermatitidis</i>
<i>P. suttonii</i>	<i>Xylohypha</i>
	<i>X. bantiana</i>
	<i>X. emmonsii</i>

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Mycology of Indoor Air

The basic principles of fungal aerobiology must be applied to the 0-g environment of Space Station *Freedom*. Microgravity, as opposed to the restricted space, will be the key issue onboard *Freedom*. In microgravity, the morphological form exhibited by fungi may be significantly different from that expressed on Earth, making the identification of the fungi on SSF extremely difficult. Owing to the biological stress fungi will experience in 0-g, physiological parameters will probably vary enough to cause changes in sporogenesis and conidiogenesis, metabolism, and the production of mycotoxins. If sexual reproduction occurs more frequently, then there will be greater recombination of genetic material.

Fungal proliferation can be controlled by lowering the relative humidity to 50% or less and preventing condensation on surfaces. In reality, this may not be a practical solution to the potential problem of fungal growth on *Freedom*. Unlike Earth systems, fungi present in 0-g will remain in an aerosolized state, and probably will become associated with floating water droplets and particulate matter in the air.

Monitoring and collection protocols will be important steps in evaluating the air environment for the presence of fungi. A particulate sampler should be considered as a means of sampling for the presence of airborne fungi. The presence of viable fungi can be inferred from particulate collections. Use of cultural sampling for fungi is not recommended due to the strong potential for aerosolization of viable spores. Total fungal counts include both viable and dead fungi, both of which may be important sources of antigens that can produce allergic reactions. Sampling for mycotoxins must include a means of detecting the toxin itself rather than the fungus that produces the mycotoxin. It is recommended that toxicological analysis of air should be expanded to include microbial volatile organic compounds that are known to be produced as a result of fungal metabolism.

The HEPA filters, as a component of the air handling system, could serve as a sampler for fungi and their metabolites. Such an approach requires detailed information regarding air circulation patterns, occupational patterns, and activity levels and patterns. Without this type of data, it is premature to determine standards for sampling frequencies and locations.

The biological safety containment cabinet will probably become contaminated by moulds over time. Of special concern would be fungi like *Aspergillus fumigatus*, which grows on bacteriological culture media. The problem will depend upon how the cabinet is used and what its principal functions are.

Fungi in the atmosphere of SSF may cause hypersensitivity disease. Many fungi can serve as potential antigens that produce hypersensitivity pneumonitis, a seriously debilitating condition that can cause permanent lung damage and death. Risk factors for the development of hypersensitivity pneumonitis are unknown. Fungal antigens also cause allergic asthma and rhinitis, both of which can seriously compromise humans.

Bacterial endotoxins pose special problems. On Earth, acceptable guidelines for airborne endotoxins are different for outdoors as compared to indoors. For example, 0.2 ug/m³ is considered an acceptable limit for outdoor endotoxins, and 4 ug/m³ for indoors. There is no reference point for correlating toxins in the SSF environment. Endotoxin acts as an adjuvant and may increase the risk for development of

hypersensitivity pneumonitis, making the combination of excessive airborne endotoxin and fungus propagules potentially deadly. Airborne endotoxins also produce serious flu-like symptoms (fever, chills, malaise) at high concentrations.

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Environmental Microbiology

The inaccessibility of the environment to be studied (i.e., space) is a major problem in predicting which microorganisms will colonize SSF. Topics to be considered should include bioremediation of waste products, especially in the lunar and Mars programs, biologically activated carbons to reduce the amount of organic carbon sources that could serve as nutrients, and removal of volatile toxic products produced by fungi and other organisms. Applying tracer technology to the problem of understanding how and where microparticulate materials distribute themselves in 0-g would be a useful approach.

A number of studies are needed in order to better understand and predict the microbial problems that will probably develop. Selective pressures in microgravity will have a profound effect on microbial morphology, physiology, pathogenicity, and formation of biofilms, as well as sensitivity to antibiotics and disinfectants.

The Vitek microbial identification and susceptibility testing system must be evaluated carefully before use on SSF. Organic toxins of microbial origin in water might be detected by using biosensors. A great deal of work will be required in evaluating the usefulness of this type of problem-solving approach. Identifying cleaning agents appropriate for use on SSF will require a great deal of basic investigative work. New solvents that are less toxic and more biodegradable than traditional solvents are presently being tested by the U.S. Air Force. Using a hygroscopic cloth impregnated with a safe effective disinfectant may be a simple way to clean surfaces.

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Biofilms and Water Supply Systems

Data from Earth-based systems demonstrate that coliform bacteria survive many disinfection processes. In space, coliforms will probably be important, but other bacteria may actually be more important, particularly those that occur as biofilms or as contaminants of the SSF water supply systems.

Potential problems will probably include minimal barriers for the elimination of bacteria, process problems involving on-off backwashing of the water supply system, and inadequate disinfectants. Other problems may include chlorine demand in grey water, interrupted treatment, inadequate disinfection concentration or contact time (C·T values), and reverse-osmosis membrane degradation by microbial activity.

Some fundamental questions to be addressed include determining the health significance of coliforms, as well as methods of suppression, particularly when they occur as biofilms. The answers to these questions are not clear. Because of the uniqueness of 0-g and space, questions such as these assume even greater importance.

Housekeeping procedures in space will be a difficult problem, especially the removal of particulate matter from the water supply systems. It will be important to maintain the integrity of the compartments that constitute the system, as well as reducing turbidity, because particulates can serve as carriers for bacteria. Similarly, crossflow and backflow can contribute to serious contamination problems. Large numbers of microorganisms can interfere with microbial population studies, though this will be unlikely when microorganisms occur at 10 CFU/ml or less. Bacteria that clump deserve special attention, as clumps may survive some disinfection processes. "Carbon fines" may also serve as vehicles for bacterial transport.

Water temperature will be extremely important. An increase of 10°C in a cold water system will increase the growth rate of bacteria significantly. Because stagnation of the water flow can produce problems, it is important that the water in the system be constantly moving. Dead microorganisms in the system can provide nutrients for further bacterial growth, although assimilable organic carbon (AOC) absorption to pipe surfaces will probably be a more realistic problem.

Bacteria will probably employ various protective mechanisms in space. Species of *Klebsiella* and *Enterobacter* will encapsulate, which will probably protect them against disinfection processes, particularly chlorine. Variations in cell metabolic rates may also be protective. Biofilm communities will provide mutual protection via slime formation and nutrient sharing. Flushing and cleaning the water supply system will minimize the formation of biofilms.

In order to reduce the potential for microbial contamination of the water supply systems, certain steps must be taken. It will be extremely important to maintain constant positive pressure in the system. Power expenditure must be evaluated in terms of the number of organisms killed. The system must be flushed in order to eliminate microorganisms. Of special concern will be the potential for extremely tolerant microorganisms to develop through mutation.

Charles P. Gerba, Ph.D.
University of Arizona

Microbial Risk Assessment

The spectrum of potentially dangerous water-borne pathogens, including nearly 130 viral agents, is constantly expanding in number. A risk-balance study approach is required to establish standards for SSF. In Arizona, recycling grey water requires that fecal coliforms not exceed 25 CFU per 100 ml of water sampled. The tolerance for viruses and parasites is zero. When water is collected in a sump and then pumped to a treatment plant, a continuous culture situation is established. Point-of-use purifiers are employed as a means of reducing the microbial risk.

Viruses and parasites have low infective dose levels (1-10 units) in comparison to *Escherichia coli* (10⁷ CFU). Although the virus level should be zero, this is a very difficult standard to reach. As an example of the importance of viruses, one ill individual on SSF could contaminate the water supply system with tremendous numbers of viral particles. The above values for microbial risk assessment refer to situations dealing with healthy, noncompromised individuals.

The microbial risk-assessment process involves several steps, including (1) development of standards and criteria; (2) determining sensitivity of analytical methods; (3) determining treatment requirements; and (4) using a cost-benefit analysis. Additional

data such as hazard identification, dose-response assessment, exposure assessment, and risk characterization are extremely important.

For a successful microbial risk assessment program, the treatment requirement for a given risk of infection involving a contaminated water supply must be determined. If all of the microbial contamination cannot be removed, standards for an acceptable level following treatment must be developed. To avoid toxicological problems, the type and amount of disinfectant must be selected carefully so that the microbial load in the water supply system is neutralized.

Helen Lucia, M.D.
University of Texas Medical Branch

Bacteriology of Air, Internal Surfaces, and Water

The majority of bacteria found in the SSF environment will originate from human skin. During a 14-day period, approximately 2 m² of epidermis is shed with its associated bacteria, e.g., *Staphylococcus epidermatitis*, *Micrococcus* spp., and diptheroids.

Males have approximately a 10-fold greater concentration of skin bacteria than do females. Gram negative bacteria tend to be associated with moist sites such as the groin. Bacteria such as *S. aureus* are often spread by inhalation of skin flakes. Vigorous activities, friction associated with clothes, and showering increase the shedding of bacteria. Bacterial die-off (e.g. *S. aureus*) occurs rapidly in environments not inhabited by humans, whereas spore-forming bacteria like *Bacillus* spp. remain viable for long periods of time.

Surfaces and air in the SSF will harbor essentially identical bacteria that originated from human skin. Organisms will be transported from surface to surface through hand and body contact. Surfaces will be extremely difficult to keep clean. Fabrics such as cotton and wool will tend to pull water from the air, thereby increasing the potential for colonization and subsequent proliferation of bacteria. Plans for cleaning must include a means of removing the large amounts of skin that will be shed. A clothes washer and dryer will be extremely important in reducing the numbers of microorganisms present. The degree of cleaning will determine the amount of organisms potentially present on surfaces and in the air.

The shower stall design being proposed for SSF presents special problems, since it appears that the system will be difficult to keep clean. In order to reduce microbial proliferation, and reduce the possibility of bacteria such as *Legionella* spp. becoming a problem, the hot water heaters must bring the water temperature up to standard.

The water system should be tested for the presence of *Mycobacterium* spp. On Earth, almost all contamination problems in hospitals can be traced to the institution's water supply system. Some organisms will grow in almost any liquid, including iodine. A great deal of thought must be devoted to the issue of disinfectants.

Microorganisms associated with the nose and throat must also be considered, particularly in light of possible immunocompromise states arising from living in microgravity.

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University of California at Irvine

Environmental Effects on Microorganisms

Microorganisms will develop adaptive responses to the aquatic environment of the water supply systems in SSF. Because of the chemical content of the water, surface tension, and bacterial proliferation, colonization of the water supply system will occur regardless of the disinfectant employed. The formation of particles, and particle-bacteria aggregates, is an important adaptive process. It is unknown whether 0-g will inhibit or enhance bacterial clumping. Adherent clusters of cells tend to be more resistant to disinfectants than cells in a planktonic stage.

Carbon, nitrogen, and phosphorus are important nutrients that will probably be available in the water supply system. Simple processes such as degassing EVA suits may provide nutrients to the environment that can be used for microbial proliferation. Bacterial metabolism will likely change depending upon the nutrients present. Phenotypic changes following adaptation to the stress environment in SSF may include changes in bacterial susceptibility, i.e., resistance to disinfectants. Such changes may occur at the genomic level.

In order to enhance the efficiency of the disinfection protocols, bacterial cells should be destroyed while they are in a planktonic stage. The physics of biofilm layer formation may be important in understanding biofilm formation and attachment characteristics. This may be critical for developing effective disinfection protocols, especially in microgravity.

Navin K. Sinha, Ph.D.
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Fidelity of DNA Replication

Of special concern in the microgravity of space will be the genetic stability of microorganisms introduced into the SSF environment. It must be expected that genomic changes will occur in space, resulting in altered microorganisms. On Earth, the mutation load per genome is relatively constant regardless of the organism. A rate of 10^{-5} , which is approximately one mistake per 1000 genomes, is normal except for more advanced animals, where a frequency of 1 mistake per 1 to 10 genomes often occurs. Enzymes that serve as "biological editors" are active at different sites of discrimination. Bacteriophages serve as agents of infection in bacterial cells. During the replication of their DNA, discrimination occurs during nucleotide insertion and subsequent editing. A third stage, postreplication mismatch correction, occurs in bacteria and possibly in higher organisms.

In space, the effect of radiation upon DNA integrity and accuracy of replication will be important. Quantifying the amount of radiation received by individuals and examining the possible effects of the radiation on human DNA and that of their offspring must be planned. Studying in vitro models using human cell lines and then extrapolating the data to the human genome is a means of understanding and defining potential hazards. Shielding for SSF should be seriously considered. Because microorganisms will be exposed to radiation that will probably cause mutations, automated microbial systems such as the Vitek instrument may not provide reliable data in space.

Infections in Compromised Hosts

Individuals are protected against infection by a number of mechanisms, including mechanisms such as phagocytic cells, complement amplification of the humoral immune system resulting in antigen-antibody complexes, lymphocytes, and mechanical barriers such as gastric acid, skin, cilia, and the cough reflex. Changes in the normal flora resulting from use of antibiotics may result in new organisms causing infection. Vascular insufficiency may cause loss of the protective barrier. Traumatic injury could produce the same effect.

The immunocompromised state need not result from dramatic processes such as cancer, but may occur as a result of burns, pregnancy, phagocytic dysfunction, splenectomy, or even as a side effect of antibiotics used to treat fever in patients that are neutropenic. Because space travel will be stressful to an individual's immune system, the normal defense barriers may be compromised even in relatively healthy people.

Measures that can be used as a means of minimizing the compromised state include eating cooked foods to avoid viable microorganisms, using electric razors, applying povidone iodine to the axilla, avoiding axillar shaving, avoiding the use of indwelling catheters, having influenza, pneumococcal, and other vaccinations, avoiding occlusive clothing, bathing and shampooing daily, following accepted hand-washing practices when examining individuals, and using oral hygiene aids like mouth washes. These, as well as other precautions, such as isolating prospective space travelers from contact with others before space flight for a duration commensurate with the incubation period of most infectious diseases, can contribute to maintaining a noncompromised state.

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Centers for Disease Control

Environmental Parasitology

Approximately 50 parasites are known agents of human disease. Free-living opportunists like water-borne species of *Entamoeba*, *Giardia*, *Cryptosporidium*, *Acanthamoeba*, *Naegleria*, *Hartmanella* and *Enterobius vermicularis* (pinworms) are of potential importance on SSF. Amoebae live in a wide variety of environments such as hot tubs, carpets, and contact lens solutions.

Naegleria fowleri and *Acanthamoeba* spp. are known to cause meningitis or even encephalitis if organisms enter through the nose and travel up the olfactory tract. These organisms may also cause pneumonitis and humidifier fever. *Acanthamoeba* are known agents of keratitis. *Giardia*, which is a water-borne organism, frequently causes infection in individuals who consume unfiltered water. While this organism is easily killed in water that is heated to 55-60°C for one minute, studies have shown that it can survive on carpets for up to 43 hours.

Amoebic infections are extremely difficult to diagnose. Immunofluorescence, although an excellent means of detection, would probably not be practical for SSF because of the low probability of parasitic diseases. Water-borne parasites like *Giardia* should be considered as potential problems, but may not be important in reality.



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16. Abstract This panel discussion convened in Houston, Texas, at the Lunar and Planetary Institute, on November 6-8, 1989, to review NASA's plans for microbiology on Space Station <i>Freedom</i> . A panel of distinguished scientists reviewed, validated, and recommended revisions to NASA's proposed acceptability standards for air, water, and internal surfaces on board <i>Freedom</i> . Also reviewed were the proposed microbiology capabilities and monitoring plan, disinfection procedures, waste management, and clinical issues. In the opinion of this advisory panel, ensuring the health of <i>Freedom</i> crews requires a strong goal-oriented research effort to determine the potential effects of microorganisms on the crewmembers and on the physical environment of the station. Because there are very few data addressing the fundamental question of how microgravity influences microbial function, the panel recommended establishing a ground-based microbial model of <i>Freedom</i> , with subsequent evaluation using in-flight Shuttle data. Sampling techniques and standards will be affected by both technological advances in microgravity-compatible instrumentation, and by changes in the microbial population over the life of the station.					
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